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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003905683 for a patent by VIP DEVELOPMENT PTY LTD as filed on 16 October 2003.

I further certify that the name of the applicant has been amended to VIRAX DEVELOPMENT PTY LTD pursuant to the provisions of Section 104 of the Patents Act 1990.

WITNESS my hand this
First day of November 2004

A handwritten signature in black ink, appearing to be 'L. Mynott'.

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES



Regulation 3.2

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PROVISIONAL SPECIFICATION
for the invention entitled:

"A viral vector and methods of using same"

The invention is described in the following statement:

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A VIRAL VECTOR AND METHODS OF USING SAME

BACKGROUND OF THE INVENTION

5 FIELD OF THE INVENTION

The present invention relates generally to a recombinant vector and its use in the treatment and/or prophylaxis of retroviral infections and the symptoms associated therewith. More particularly, the present invention provides a recombinant vector for
10 use in conjunction with anti-retroviral drug treatment (ARDT) to modulate viral load in a subject. The present invention specifically relates to a recombinant poxvirus vector expressing a retrovirus antigen and/or a modulatory factor and its use in conjunction with anti-HIV retroviral drug therapy in the treatment or prophylaxis of HIV infection, AIDS and AIDS-related disorders in a human subject. The vectors and methods of the
15 present invention are particularly useful in preventing, reducing or delaying viral rebound when retroviral therapy is interrupted.

20 DESCRIPTION OF THE PRIOR ART

Bibliographic details of the references in this specification are collected at the end of the description.

Reference to any prior art in the specification is not, and should not be taken as, an
25 acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Retroviruses are obligate intracellular parasites of vertebrate cells. Viral propagation of the enveloped RNA virus is via a double stranded DNA provirus intermediate which
30 integrates into the genomic DNA of a susceptible host cell and makes use of many host cell factors. This efficient system of infection and propagation makes eradication of the virus very difficult. It is estimated that HIV replication in an infected individual can

5 All retrovirus genomes comprise three major coding domains: *gag*, which is responsible for matrix, capsid and nucleoprotein structures; *pol* which encodes are RNA-dependent DNA polymerase, reverse transcriptase, and also integrase enzymes; and *env* which generates viral envelope proteins. In addition, all retroviruses also comprise the *pro* coding domain responsible for producing virion protease. A subset of retroviruses termed

10 "complex" retroviruses also comprise a range of regulatory factors which influence their own and host expression pathways.

The retrovirus family includes Lentiviruses such as Human immunodeficiency virus (HIV-1 and HIV-2), Simian immunodeficiency virus (SIV), Human T-cell leukaemia-bovine leukaemia viral group such as Human T-cell leukaemia virus (HTLV), Feline leukaemia virus (FLV) and Spumaviruses as described in Vogt P.K. (Chapter 1: *Retroviruses*: Coffin John M et al (eds), Cold Spring Harbour Laboratory Press, USA, 1997).

20 HIV is a particularly important complex retrovirus of humans as the causative agent of Acquired Immune Deficiency Syndrome (AIDS) which remains a devastating and complex problem despite recent advances in anti-retroviral drug treatments.

HIV infects CD4+ immune cells and established HIV infections are characteristically associated with progressive immune system damage, opportunistic infections and wasting syndromes. Commencement of anti-retroviral therapy is generally recommended at any stage of HIV infection when immune deficiency is present as determined by, for example, low levels of CD4+ cells. Reductions in plasma viral load in response to anti-retroviral treatment are associated with statistically significant improvements in survival and clinical outcome (Melors J.W. *et al*, *Science* 272:1167-1170, 1996). Complete eradication of HIV in a subject is presently considered to be an unrealistic goal, and as viral levels may

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There are a large range of anti-retroviral drug treatment regimens involving the administration of combinations of anti-retroviral compounds (see for example ASHM-2001 draft Australian antiretroviral guidelines, *supra*). In particular, limited clinical data have indicated that triple therapy in the treatment of acute and advanced HIV infection employing a nucleoside analogue combination and a non-nucleoside reverse transcriptase inhibitor or protease inhibitor has a positive effect on surrogate markers of disease progression and at least a short term clinical benefit.

The optimal regimens and timing for anti-retroviral treatment are unclear. The emergence of drug resistant strains is a major problem contributing to drug treatment failure. Compliance is also a major problem because anti-retroviral drug treatment regimens are characteristically complex and require strict adherence in order to have any chance of success. Current regimens often involve multiple dosings of up to four different active agents. Each active agent typically has its own administration requirements, for example administration before or after food. Similarly each agent will need to be administered in specified quantities at specified periods, such that the patient will frequently be taking, for example, one medication 4 times a day, another 3 times a day and another twice a day, with one needing to be taken before food and one needing to be taken after food. In addition the common side effects of anti-retroviral drug treatment include nausea, vomiting, heart disease, diabetes and liver damage.

25 In view of the difficulties associated with anti-retroviral drug treatment there is an urgent need for greater understanding of the host-retrovirus interaction and to identify effective methods and reagents for controlling retroviral infections and improving current anti-retroviral drug treatment regimens particularly to facilitate their long term efficacy. Also, in view of the undesirable and often severe side-effects, there is a need for treatment
30 protocols which allow periods in which anti-retroviral drugs are not administered. As a

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result of the onerous and intrusive treatment regimens, there is a demand from patients for protocols which allow them periods in which they do not take anti-retroviral drugs.

5 SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the
10 exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of sequence identifiers is
15 provided in Table 1. A sequence listing is provided after the claims.

The present invention is predicated, in part, on the development of a vector which expresses a retrovirus antigen and/or a host modulatory factor and which upon administration to a subject is capable of reducing, preventing or delaying viral rebound or
20 of reducing, preventing or delaying the increase or rate of increase in viral load in a subject. As there are significant disadvantages and difficulties with present anti-retroviral drug treatment regimens in terms of their efficacy, side effects and compliance, it is anticipated that the vectors of the present invention will find broad application in the treatment of retroviral infections in conjunction with anti-retroviral drug treatment.

25

In one aspect, the present vector is a poxvirus vector which expresses one or more antigens of HIV and/or a cytokine which vector is administered in conjunction with anti-retroviral drug therapy. In a preferred aspect the present invention provides a method for treatment or prophylaxis of one or more symptoms of retroviral infection such as HIV infection,
30 comprising the administration of a poxvirus vector encoding a retrovirus antigen and/or a cytokine or a functional homolog, derivative, part or analog thereof, in conjunction with

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anti-retroviral drug therapy wherein said polypeptide and/or cytokine are expressed in a subject and are effective in maintaining a low viral load in a subject for a period of time, for example effectively preventing, reducing or delaying viral rebound during interruption of anti-retroviral drug treatment. Methods are also provided for reducing or alleviating one or more of the side effects of ARDT comprising administering the instant vectors for a time and under conditions to reduce or alleviate one or more of the said effects of ARDT. The vectors may be administered before and/or during ARDT and/or after withdrawal of ARDT. In an exemplary embodiment, the vector is a fowlpox vector co-expressing gag/pol and IFN γ which effectively maintains a low viral vector load, or delays the increase in viral load when antiretroviral drug treatment is interrupted. The present invention extends to pharmaceutical agents comprising the vectors of the present invention and their use in a range of treatment regimens.

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A summary of sequence identifiers used throughout the subject specification is provided in Table 1.

TABLE 1**Summary of sequence identifiers**

SEQUENCE ID NO.	DESCRIPTION
1	Nucleotide sequence encoding HIV gag
2	Amino acid sequence encoded by SEQ ID NO: 1
3	Nucleotide sequence encoding HIV pol
4	Amino acid sequence encoded by SEQ ID NO: 3
5	Nucleotide sequence encoding human IFN γ
6	Amino acid sequence encoded by SEQ ID NO: 5
7	Nucleotide sequence of insertion site of rFPV gag/pol IFN γ

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graphical representation showing the mean viral load (non-log) over the 20 week period of the extension trial for each vector recipient group. Subject Group A (white line) received the full construct (FC) comprising recombinant FPV expressing HIV-1 gag/pol and interferon-gamma (IFN γ). Subject Group B (black line) received the partial construct (PC) comprising recombinant FPV expressing HIV-1 gag/pol. Subject Group C (grey line) received diluent alone (placebo).

Figure 2 is a graphical representation showing the proportion of recipients in each recipient group whose viral load was low enough over the period of the study (in days) such that ARDT was not re-initiated. Subject Group A received the full construct (FC) comprising recombinant FPV expressing HIV-1 gag/pol and interferon-gamma (IFN γ). Subject Group B received the partial construct (PC) comprising recombinant FPV expressing HIV-1 gag/pol. Subject Group C received diluent alone (placebo).

Figure 3 is a representation of the nucleotide sequence of the insertion site of the recombinant FPV gag/pol IFN γ employed in the Examples.

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DETAILED DESCRIPTION OF THE INVENTION

5 The present invention provides a vector which effectively modulates retroviral load in a subject. Specifically, the vector of the present invention maintains or prolongs a low viral load in a subject infected with a retroviral infection. In a preferred aspect the vector of the present invention is used in conjunction with anti-retroviral drug therapy and is useful in maintaining a low viral load before, after or between periods of drug therapy.

10 In one aspect, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and/or a sequence of nucleotides encoding a modulatory factor, or a functional homolog, derivative, part or analog thereof, which expresses said sequences for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

15 Reference herein to anti-retroviral drug treatment (ARDT) is used in its broadest context to include the use of one or more compounds, singly or in combination in regimens for retroviral, and in particular HIV retroviral, treatment.

20 Anti-retroviral compounds act by a number of different of mechanisms which selectively affect the virus. For example, protease inhibitors, reverse transcriptase inhibitors and ribonucleotide reduction inhibitors may be employed or compounds which inhibit viral adsorption, assembly, integration and transcription. As will be known to those skilled in the art there are a large number of anti-retroviral compounds which may be administered.

25 Examples of protease inhibitors include Indinavir and Nelfinavir. Reverse transcriptase inhibitors include, for example, Zidovisidine, Stavudine and Didanosine. Examples of ribonucleotide reductase inhibitors include thiosemicarbazone derivatives.

The particular compounds and combinations used and the dosages and regimens will be determined by the administering practitioner and will depend *inter alia*, upon individual responses to the treatment.

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In another aspect, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a modulatory factor, or a functional homolog, derivative, part or analog thereof, which co-expresses said constituents for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

As used herein the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to a "compound" includes a single compound, as well as two or more compounds; reference to "an active agent" includes a single active agent, as well as two or more active agents; and so forth.

The term "antigen" is used in its broadest context to include molecules comprising one or more epitopes against which an immune response is produced. The term however, also includes within its scope any polypeptide, including a protein or peptide. Antigenic portions may be identified using well known techniques, such as those set out in Paul, Fundamental Immunology, 3rd Ed., 243-247 (Raven Press, 1993) and references cited therein.

The term "recombinant vector" is used herein in its broadest sense as a reference to constructs which are capable of vectoring or carrying nucleic acid molecules into a target cell for expression therein. The vectors of the present invention include viral vectors or similar constructs or derivatives thereof, plasmid vectors or naked nucleic acid molecules.

Poxvirus vectors are particularly convenient vectors. As used herein reference to "poxvirus" includes viruses selected from the group comprising avipox (eg, fowlpox, canarypox, pigeonpox) orthopox (eg, vaccinia) capripox (eg, sheep, goats) and suipox (eg, swinepox). Preferred poxvirus vectors are avipox or orthopox vectors. Avipox vectors are preferred vectors. A particularly preferred avipoxvirus vector is a fowlpox vector (FPV). Exemplary fowlpox vectors are FPV-M3 vectors as described in International Patent Publication No. WO 00/28003. The principles and procedures for

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generating and using recombinant poxvirus vectors are well known in the art. Briefly, homologous recombination between a donor recombination vector and a poxvirus within a host cell permits correct introduction of the desired sequences.

- 5 Reference to "modulates" includes down regulations of viral load, maintenance of viral load and a change in the rate of increase of viral load. Specifically, any change in viral load is usually but not exclusively determined over an appropriate period of time and is expressed in terms of change in average viral load over time of a subject or group of subjects.

10

Accordingly, the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a modulatory factor, or a functional homolog, derivative, part or analog thereof, which co-expresses said constituents for use in conjunction with ARDT in the treatment or

- 15 prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

Reference to "treatment" and "prophylaxis" are to be considered in their broadest context. The term treatment includes partial and full recovery of HIV infection or of the clinical symptoms of AIDS. The term "prophylaxis" includes a delay in contracting an HIV

- 20 infection or experiencing symptoms of HIV infection including the clinical symptoms of AIDS. Certain symptoms are shared between symptoms of an HIV infection, and the clinical symptoms of AIDS. As will be understood by one skilled in the art, examples of shared symptoms include a detectable viral load and reduced levels of CD4+ cells. Certain HIV infected individuals have a low viral load and fail to show the clinical symptoms of
- 25 AIDS such as immunosuppression, wasting diseases or increased levels of opportunistic infections. Accordingly, the vectors of the present invention are used to treat the symptoms of HIV infection and/or than the clinical symptoms of AIDS and AIDS related disorders.

- 30 Although human subjects are primarily contemplated, reference to a "subject" should be understood to include mammals including primates (eg, humans, monkeys), livestock

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animals (eg, sheep, cows, horses, donkeys, goats, pigs), laboratory test animals (eg, mice, rats, ducks, dogs, guinea pigs, rabbits, hamsters), companion animals (eg, dogs, cats, birds), and captive wild animals (eg, kangaroos, deer, foxes). Preferably said subject is a mammal, more preferably a primate and even more preferably a human.

5

The phrase "modulatory factor" is used herein include to those host factors which act as chemical messengers between cells to effect change in response to external or internal stimuli. Thus ligands for cellular receptors such as cytokines, growth factors and chemokines are contemplated together with their functional homologs, parts, derivatives and analogs. As will be understood by those skilled in the art, the activity of such a factor may also be achieved through the administration of a compound which acts as an agonist of said factor or as an antagonist of inhibitors of the said factor or by down stream effectors in the same pathway or network. Accordingly, the term modulatory factors includes reference to the host factor, its down stream effectors and agonists thereof.

15

In a further embodiment, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional homolog, derivative, part or analog thereof, which co-expresses said sequences for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

20

In a further embodiment, the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional homolog, derivative, part or analog thereof, which co-expresses said sequences for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

25

In a particularly preferred embodiment, the modulatory factor of the present invention is selected from IFN γ , IL-12, IL-2, TNF and IL-6 and down stream effectors and agonists

30

In a preferred, aspect the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding IFN γ , or a functional homolog, derivative, part or analog thereof, which co-expresses said constituents for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms of a retroviral infection in a subject.

Particularly preferred antigens are those encoded by *gag* and/or *pol* coding regions. A *gag/pol* construct is also preferred.

The present invention is particularly directed to the treatment of human retroviral infections such as HIV and preferably HIV-1.

Accordingly, in another preferred aspect the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding gag and/or pol antigens from HIV and a sequence of nucleotides encoding IFN γ , or a functional homologue, derivative, part, or analogue thereof, which vector co-expresses said sequences for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms of an HIV infection or AIDS in a subject.

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analogue thereof, which vector co-expresses said sequences for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms of an HIV infection or AIDS in a subject.

- 5 Preferably said poxvirus is a fowlpox virus.

In a further embodiment, the gag antigen is encoded by a sequence of nucleotides set forth in SEQ ID NO: 1 or a sequence of nucleotides having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

In a further embodiment, the gag antigen is comprises a sequence of amino acids set forth in SEQ ID NO: 2 or a sequence of amino acids having at least 60% similarity thereto after optimal alignment.

15 In a further embodiment, the pol antigen is encoded by a sequence of nucleotides set forth in SEQ ID NO: 3 or a sequence of nucleotides having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

20 In a further embodiment, the pol antigen is comprises a sequence of amino acids set forth in SEQ ID NO: 4 or a sequence of amino acids having at least 60% similarity thereto after optimal alignment.

25 In a further embodiment, the IFN γ antigen is encoded by a sequence of nucleotides set forth in SEQ ID NO: 5 or a sequence of nucleotides having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

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In a further embodiment, the IFN γ antigen is comprises a sequence of amino acids set forth in SEQ ID NO: 6 or a sequence of amino acids having at least 60% similarity thereto after optimal alignment.

- 5 In a further embodiment, the vector is fowlpox vector comprising the nucleotide sequence set forth in SEQ ID NO: 7 or a sequence of nucleotides having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.
- 10 SEQ ID NO: 7 provides the sequence of the insertion site of rFPV gag/pol IFN γ .

A "functional homolog" include species homologs whose function is conserved between species. Thus a functional homology of IFN γ retains its modulatory function. A functional homolog of pol, for example, retains its antigenic or biochemical function.

15

A "functional derivative" of an antigen or modulatory factor encompasses variants and portions or a part of a full length polypeptide, which retains the functional activity of the parent molecule. Such, active fragments include deletion mutants and small peptides, for example, of at least 10, preferably at least 20 and more preferably at least 30 contiguous

20 amino acids, which exhibit the requisite activity. Peptides of this type may be obtained through the application of standard recombinant nucleic acid techniques or synthesized using conventional liquid or solid phase synthesis as described in Chapter 9 entitled "Peptide Synthesis" by Atherton and Shephard which is included in a publication entitled "Synthetic Vaccines" edited by Nicholson and published by Blackwell Scientific

25 Publications.

The term "functional" means that the molecules retain or exceed the overall function of the parent. Accordingly, if in particular function is diminished in the derivative or homolog, this is compensated for new functions such as, for example, greater antigenicity, longevity,

30 half-life, activity, avidity etc.

The term "variant" refers to nucleotide sequences displaying substantial sequence identity with a reference nucleotide sequences or polynucleotides that hybridize with a reference sequence under stringency conditions that are defined hereinafter. The terms "nucleotide sequence", "polynucleotide" and "nucleic acid molecule" may be used herein interchangeably and encompass polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference nucleotide sequence whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide.

The term "variant" also includes naturally-occurring allelic variants.

Functional derivatives of a target molecule include active portions of the target molecule whose modification in a subject ameliorates a disease or condition and which may be further modified to enhance this affect. A functional derivative of a target molecule in the form of a protein or peptide comprises a sequence of amino acids having at least 60% similarity to the target molecule or portion thereof. A "portion" in peptide form may be as small as an epitope comprising less than 5 amino acids or as large as several hundred kilodaltons. The length of the polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues and preferably more than about 35 residues.

When in nucleic acid form, a functional derivative comprises a sequence of nucleotides having at least 60% similarity to the target molecule after optimal alignment. A "portion" of a target nucleic acid molecule is defined as having a minimal size of at least about 10 nucleotides or preferably about 13 nucleotides or more preferably at least about 20 nucleotides and may have a minimal size of at least about 35 nucleotides. This definition includes all sizes in the range of 10-35 nucleotides including 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleotides as well as greater than 35 nucleotides including 50, 100, 300, 500, 600 nucleotides or nucleic acid molecules having any number of nucleotides within these values.

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Functional derivatives of target molecules in nucleic acid form include nucleic acid molecules comprising a nucleotide sequence capable of hybridising to the target molecule or its complementary form under low stringency conditions.

5

Analogues contemplated herein include but are not limited to modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

10

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; 15 trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

20 The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitization, for example, to a corresponding amide.

25

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4- 30 chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-

chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

5 Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by
10 alkylation with iodoacetic acid derivatives or N-carbethoxylation with
diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 2.

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TABLE 2

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5	α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
			L-N-methylaspartic acid	Nmasp
10	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
			L-N-methylglutamic acid	Nmglu
	cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Das	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmtur
25	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbg
30	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva

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	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgabu
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
5	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpcn
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Nebut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- α -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
30	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis

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	D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
5	N-methylglycine	Nala	D-N-methylphenylalanine	Dmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
10	D-N-methyltyrosine	Dnmtyr	N-methyl-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
15	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mglu	L- α -methylglutamate	Mglu
20	L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
	L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
	L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
25	L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
	L- α -methylserine	Mser	L- α -methylthreonine	Mthr
	L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
	L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhpe
30	N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
	carbamylmethyl)glycine		carbamylmethyl)glycine	

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1-carboxy-1-(2,2-diphenyl-N-mbo
ethylamino)cyclopropane

- 5 Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or
- 10 carbodilimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α -methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

15

These types of molecules may be important to stabilise vector constructs or their expressed products.

- The terms "similarity" or "identity" as used herein include exact identity between
- 20 compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the
- 25 structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and amino acid sequence comparisons are made at the level of identity rather than similarity.

- Terms used to describe sequence relationships between two or more polynucleotides or
- 30 polypeptides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", "percentage of sequence similarity", "percentage of sequence

identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as, for example, disclosed by Altschul *et al.* (*Nucl. Acids Res.* 25: 3389, 1997). A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.* ("Current Protocols in Molecular Biology" John Wiley & Sons Inc, 1994-1998, Chapter 15).

The terms "sequence similarity" and "sequence identity" as used herein refer to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g. A, T, C, G, I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched

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10 Preferably, the percentage similarity between a particular sequence and a reference sequence (nucleotide or amino acid) is at least about 60% or at least about 70% or at least about 80% or at least about 90% or at least about 95% or above such as at least about 96%, 97%, 98%, 99% or greater. Percentage similarities or identities between 60% and 100% are also contemplated such as 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75,
15 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%.

Reference herein to a low stringency includes and encompasses from at least about 0 to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization, and at least about 1 M to at least about 2 M salt for washing conditions. Generally, low stringency is at from about 25-30°C to about 42°C. The temperature may be altered and higher temperatures used to replace formamide and/or to give alternative stringency conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41 (G+C)\%$ (Marmur and Doty, *J. Mol. Biol.* 5: 109, 1962). However, the T_m of a duplex DNA decreases by 1°C

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with every increase of 1% in the number of mismatch base pairs (Bonner and Laskey, *Eur. J. Biochem.* 46: 83, 1974). Formamide is optional in these hybridization conditions. Accordingly, particularly preferred levels of stringency are defined as follows: low stringency is 6 x SSC buffer, 0.1% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC
5 buffer, 0.1% w/v SDS at a temperature in the range 20°C to 65°C; high stringency is 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least 65°C. High stringency conditions are particularly preferred.

The present invention contemplates expression of the nucleotide sequences encoding the
10 modulatory factor and/or the retroviral antigen in recipient cells. However, appropriate alternative means to deliver said agents to recipient cells may be practiced within the scope of the present invention. Thus, the modulatory factor may be administered in proteinaceous or other suitable and pharmaceutically acceptable chemical form optionally in conjunction with the vector of the present invention comprising a nucleotide sequence encoding a
15 retroviral antigen and/or said modulatory factor.

In another aspect the present invention provides a pharmaceutical composition comprising any one of the above-described vectors together with a pharmaceutically acceptable carrier and/or diluent for use in conjunction with ARDT in the treatment or prevention of a
20 retroviral infection.

The term pharmaceutical composition is used herein to refer to a chemical compound which induces a desired pharmacological and/or physiological effect. The term encompasses pharmaceutically acceptable and pharmacologically active ingredients of the
25 active agent and includes pharmaceutically acceptable and pharmacologically active salts, esters, amides, pro-forms, metabolites, analogues, etc. The term "compound" as used herein is not to be construed as a chemical molecule only but extends to peptides, polypeptides, and proteins as well as nucleic acid molecules and chemical analogues thereof.

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By "pharmaceutically acceptable" excipient or diluent is meant a pharmaceutical vehicle comprised of material which is not biologically or otherwise undesirable, ie the material may be administered without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, colouring agents, wetting or emulsifying agents, buffering agents, preservatives, and the like.

In a preferred aspect said pharmaceutical composition is useful in conjunction with anti-retroviral drug treatment to modulate viral load in a subject.

10 In another aspect, the present invention contemplates a recombinant vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof, wherein upon administration to a subject carrying a low retroviral load, said antigen and/or cytokine is expressed in target cells and said low viral load is effectively maintained or
15 prolonged.

In another aspect, the present invention contemplates a recombinant vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analog thereof, wherein
20 upon administration to a subject carrying a low retroviral load, said nucleotide sequences are expressed in target cells and said low viral load is effectively maintained or prolonged.

Expression as used herein broadly is a reference to the production of a polypeptide from a nucleic acid molecule.

25

Viral load is measured in terms of the number of viral particles/ml of plasma and is a useful and direct measure of viral infection and a surrogate marker of efficacy in retroviral treatment regimens including drug treatments and immunisation protocols. In particular, anti-retroviral drug treatment is usually started in a patient when their viral load goes
30 above or is maintained above about 50 viral particles/ml of plasma for an appropriate period of time. One of the consequences of stopping or interrupting anti-retroviral drug

30 The delay in viral rebound or a delay in an increase in viral load is any time frame which is likely to convey clinical benefit and may be measured in days, weeks, months or years. As

5 Poxvirus vectors are particularly convenient vectors. Preferred poxvirus vectors are avipox or orthopox vectors which do not replicate efficiently in human subjects. A particularly preferred poxvirus vector is a fowlpox vector (FPV). Exemplary fowlpox vectors are FPV-M3 vectors as described in International Patent Publication No. WO 00/28003.

In a particularly preferred embodiment, the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6 and down stream effectors and agonists thereof. IFN γ is exemplified herein and IFN γ or its functional homologs, parts, derivatives and analogs are preferred.

Particularly preferred antigens are those encoded by *gag* and/or *pol* coding regions. A *gag/pol* construct is also preferred.

The preferred retrovirus is HIV-1.

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Accordingly, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof, wherein upon administration to a subject carrying a low retroviral load as a result of ARDT, said antigens
5 are expressed in target cells and said low viral load is effectively maintained or prolonged after or while ARDT is withdrawn.

For the avoidance of doubt, the instant vector may be administered before, during, after or between ARDT/s.

10

In a preferred aspect, the present invention provides a method of treatment or prophylaxis comprising the administration of a vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof in conjunction with ARDT wherein said
15 method is effective in maintaining a low retroviral load in a subject or reducing or delaying viral rebound in said subject.

In a preferred aspect, administration of the instant vector effectively prevents or treats one or more of the symptoms of HIV infection or AIDS.

20

In another aspect the present invention provides a method of treatment or prophylaxis comprising the administration of a vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analog thereof in conjunction with ARDT wherein said
25 method is effective in maintaining a low retroviral load in a subject or reducing or delaying viral rebound in a subject.

In a preferred aspect administration of the instant vectors effectively prevents or treats one or more of the symptoms of HIV infection or AIDS.

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In a related aspect, the present invention provides a method of treatment or prophylaxis of AIDS comprising the administration of a vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof in conjunction with ARDT
5 wherein said method is effective in maintaining a low retroviral load in a subject or reducing or delaying viral rebound in the absence of ARDT.

To be "effective" an "effective amount" of the instant vector is administered. As used herein an effective amount mean a sufficient amount of the vector to provide the desired
10 therapeutic or physiological outcome. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount and frequency of administration required will vary from subject to subject, depending on the species, age and general clinical condition of the
15 subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

20 The molecules of the present invention can be formulated in pharmaceutic compositions which are prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing, Company, Easton, PA, U.S.A.). The composition may contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may comprise,
25 in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g. intravenous, oral, intrathecal, epineural or parenteral. Intramuscular
30 administration is preferred.

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Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic or if it would otherwise require too high a dosage or if it would not
5 otherwise be able to enter the desired cells.

Cell based delivery system may be employed such as described in U.S. Patent No. 5,550,050 and International Patent Publication Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and
10 WO 97/12635. The vector could be targeted to cells harbouring latent infection or expression of expression products could be limited to specific cells, stages of development or cell cycle stages. The cell based delivery system is designed to be implanted in a patient's body at the desired site. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an activating agent produced in, or
15 targeted to, the cells to be treated. See, for example, European Patent Application No. 0 425 731A and International Patent Publication No. WO 90/07936.

In another aspect, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a vector
20 comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine, or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express said sequences and to reduce or alleviate one or more of the side effects of ARDT.

25 In a further aspect, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine, or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express said sequences and to
30 reduce or alleviate one or more of the side effects of ARDT.

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In another aspect, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine, for a time and under conditions sufficient to co-express
5 said sequences and to reduce or alleviate one or more of the side effects of ARDT.

Preferably, said vector is a poxvirus vector. More preferable an avipox vector. Still more preferably a fowlpox vector.

10 In a further preferred embodiment, the cytokine is INF- γ .

Preferably the retroviral antigen is *gag* and/or *pol*. Most preferably HIV *gag/pol* is employed.

15 In a further preferred embodiment, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a fowlpox vector comprising a sequence of nucleotides encoding HIV *gag/pol* and a sequence of nucleotides encoding INF- γ or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express said sequences and
20 to reduce or alleviate one or more side effect of ARDT.

The side effects of ARDT are numerous and are well known in the art and include, without limitation, nausea, vomiting, fever fat redistribution, heart disease, liver disease and insulin resistance. Treatment and prophylaxis regimens are tailored to the individual and include
25 priming and/or boosting with the vector before or during ARDT or after withdrawal ARDT and before or after re-initiation of ARDT. ARDT may be withdrawn for a period of time ranging from days to several months depending on the level and extent of side effects experienced by a recipient and the vector may be administered in prime and/or boost format during this period to maintain low level of viral load.

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In a related aspect the present invention extends to the use of the subject vectors in the manufacture of a medicament for use in conjunction with ARDT in the treatment or prophylaxis of a retroviral infection and symptoms associated therewith.

- 5 In one aspect, the present invention broadly contemplates the use of a vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine or a functional derivative, homolog, part or analog thereof in the manufacture of a medicament for use in a method of reducing or alleviating one or more of the side effects of ARDT.

10

Preferably the subject has previously been treated with an anti-retroviral compound. The instant vectors may be administering before or during ARDT or after withdrawal of ARDT. When administered before or during ARDT, ARDT may subsequently be withdrawn and in accordance with the present invention, viral loads are maintained at a

- 15 low level in the absence of ARDT.

In accordance with this aspect of the invention, preferably, said vector is a poxvirus vector. More preferably an avipox vector. Still more preferably a fowlpox vector.

- 20 In a further preferred embodiment the cytokine is IFN γ . Preferably the retroviral antigen is gag and/or pol. Most preferably HIV gag/pol are employed.

- Accordingly, in a preferred embodiment, the present invention provides the use of a fowlpox vector comprising a sequence of nucleotides encoding HIV gag/pol and a
25 sequence of nucleotides encoding IFN γ or a functional derivative, homolog, part or analog thereof in the manufacture of a medicament for use in a method of reducing or alleviating one or more of the side effects of ARDT.

- Said medicament is conveniently in a format for administration as a priming dose and/or a
30 boosting dose. A broad range of doses may be applicable. For example, a unit dose may comprise from about 1×10^6 PFU per ml to about 1×10^8 PFU per ml. Dosage regimens

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are adjusted to provide the optimum therapeutic dose and priming administrations may be administered daily, weekly or monthly or at other suitable time intervals or may be proportionately reduced as indicated by the exigencies of the situation. A preferred priming dose is 5×10^7 PFU per ml in one ml of diluent. Boosting doses may be the same
5 as priming doses or they may be more or less concentrated as indicated by the exigencies of the situation. For other constructs, from about 0.1 μ g to 1 mg of vector may be administered per kilogram of body weight per day.

The present invention is further described by the following non-limiting Examples.

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EXAMPLE 1

Randomised, Placebo-controlled, Phase I/IIa Evaluation of the Safety and Biological Activity of Avipox Virus Expressing HIV gag-pol and Interferon-gamma in HIV-1 Infected Subjects.

A clinical trial was conducted to establish the safety and immunogenicity of recombinant fowlpox virus vaccines (rFPV) expressing HIV gag-pol or co-expressing HIV gag/pol and human interferon-gamma (IFN γ) in HIV positive subjects taking combination anti-retroviral drug therapy (ART). A total of 34 patients completed the trial in which they received a series of injections and blood tests regularly over six months. Patients continued to take standard anti-retroviral therapies throughout the trial period. As announced on 17 February, 2003 (virax.com.au) the data for this trial indicated that neither construct elicited a specific immune response in trial participants receiving ART.

EXAMPLE 2

20 Safety, Biological Activity and Extension Study to Assess The Anti-retrovirological Properties of a Therapeutic HIV Vaccine Candidate Based on Recombinant Fowlpox Virus (rFPV).

A multicentre, randomised, double-blind, placebo-controlled trial recruited HIV-infected individuals treated with anti-retroviral therapy (ART) during primary HIV infection, who maintained control of virus replication (plasma viral load < 50 copies/mL) since initiation of ART. Subjects were randomised to one of three study arms: diluent alone (placebo), rFPV expressing HIV gag/pol (partial construct - PC) or rFPV expressing HIV gag/pol and IFN γ (full construct - FC). Vaccines were administered by intramuscular injection on day 0, week 4 and week 12 at a unit dose of 5×10^7 pfu/mL in 1.0mL of diluent. Follow-up continued over 52 weeks. Primary endpoints were mean change in CD8+ effector function

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as determined by CTL response or ELISPOT assay from baseline to week 26 and increase in log viral load from baseline to week 52. Analyses of safety endpoints was according to treatment received. All analyses were performed using "intention to treat" methods.

5 In this trial, 35 eligible subjects were randomised (12 placebo, 11 PC-rFPV, 12 FC-rFPV). All but one subject (placebo group) received all three immunisations. All 35 subjects completed 52 weeks of follow-up. No significant toxicity or safety concerns were observed during the study. Episodes of detectable HIV viremia (eight episodes in five patients) were infrequent across the 52 weeks of study and there was no difference between vaccine
10 groups. There were no significant differences between the combined PC and FC groups with placebo patients for anti-HIV gag ELISPOT responses (time-weighted mean difference in change from baseline = -56 sfu/106 PBMC; $p = 0.062$), anti-HIV p55 lymphoproliferative responses (time-weighted mean difference in change from baseline =
15 4.4 SI; $p = 0.337$), anti-HIV gag lymphoproliferative responses (time-weighted mean difference in change from baseline = 2.1 SI; $p = 0.778$). No additional anti-HIV antibody responses were observed during follow-up. Western Blot reactive anti-FPV antibodies were detected in all PC and FC recipients at week 6 and persisted for the duration of the study. Vaccine recipients generated long-lasting reactive anti-FPV antibodies soon after administration of candidate vaccines.

20

A pilot multicentre, double-blind, placebo-controlled 20-week extension of the study was conducted to examine the effect of immunisation with recombinant fowlpox virus vaccines (rFPV) on measures of HIV replication following cessation of combination antiretroviral therapy (ART). Previously enrolled individuals protocol were re-consented on day 0, prior
25 to receiving a boosting vaccination by intramuscular injection in accordance with their original randomised assignment: diluent alone (placebo), rFPV expressing HIV gag/pol (partial construct - PC) or rFPV expressing HIV gag/pol and interferon-gamma (full construct - FC). All ART was ceased one week following immunisation. Virological and immunological monitoring was monitored frequently for 20 weeks after immunisation. The
30 primary endpoint was time-weighted area under the curve change from plasma HIV-RNA VL (pVL) at baseline until reintroduction of ART. Secondary endpoints included log pVL

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after cessation of ART (post-vaccination pVL set-point), kinetics and rate of pVL recrudescence, median time to reinitiation of ART, CD8+ T-cell responses to HIV antigens and CD4+/CD8+ T-cell count changes.

- Twenty-five (71%) of the original study cohort consented to participate (placebo = 7; PC = 8; FC = 10). Antiretroviral therapy was re-introduced in 7 patients (placebo = 3; PC = 3; FC = 1). Immunisations were well-tolerated. One patient (PC group) experienced a transient grade 3 thrombocytopenia that resolved without treatment. The time weighted mean change from baseline pVL over 20 weeks was 1.80 (0.72), 1.78 (0.91) and 0.96 (0.91) for placebo, PC and FC respectively ($p = 0.253$, when comparing FC and PC recipients to placebo). The time-weighted mean change from baseline CD4+ cell count was -90.7 (210.1), 2.05 (166.3) and 3.45 (160.9) for placebo, PC and FC respectively ($p = 0.238$, when comparing FC and PC recipients to placebo). All patients had at least one detectable pVL (>50 copies/mL) during follow-up. FC and PC recipients compared to placebo had similar times to detectable pVL (hazard ratio 1.21, 95% CI 0.40 – 2.97, $p = 0.682$). Time to reinitiation of ART was not statistically significantly different in FC and PC recipients compared to placebo (hazard ratio = 2.08, 95% CI 0.49 – 9.31, $p = 0.338$).

Recipients of the Full construct (FC) rFPV immunization experienced a log reduction in pVL compared to recipients of the PC rFPV or placebo. Specifically, the average maximum viral loads for each of the groups was as follows: placebo group-67173 copies/ml; partial construct group-68841; and full construct group-18897 (see Figure 1). Unexpectedly therefore, notwithstanding the lack of any demonstrable immune response in the early part of the trial, in the absence of ARDT, administration of the vector resulted in an approximately 10 fold reduction in average viral load and therapeutic effect over the 20 week period of the study. As specified above, retroviral therapy was re-introduced in a total of seven patients, the seven comprising three from the placebo group, three from the group receiving the partial construct and only one from the largest group receiving the full construct as shown in Figure 2.

- 30 The nucleotide sequence of the insertion site of the vector of rFPV gag/pol IFN γ trialed in this study is set forth in Figure 3 and is represented in SEQ ID NO: 7.**

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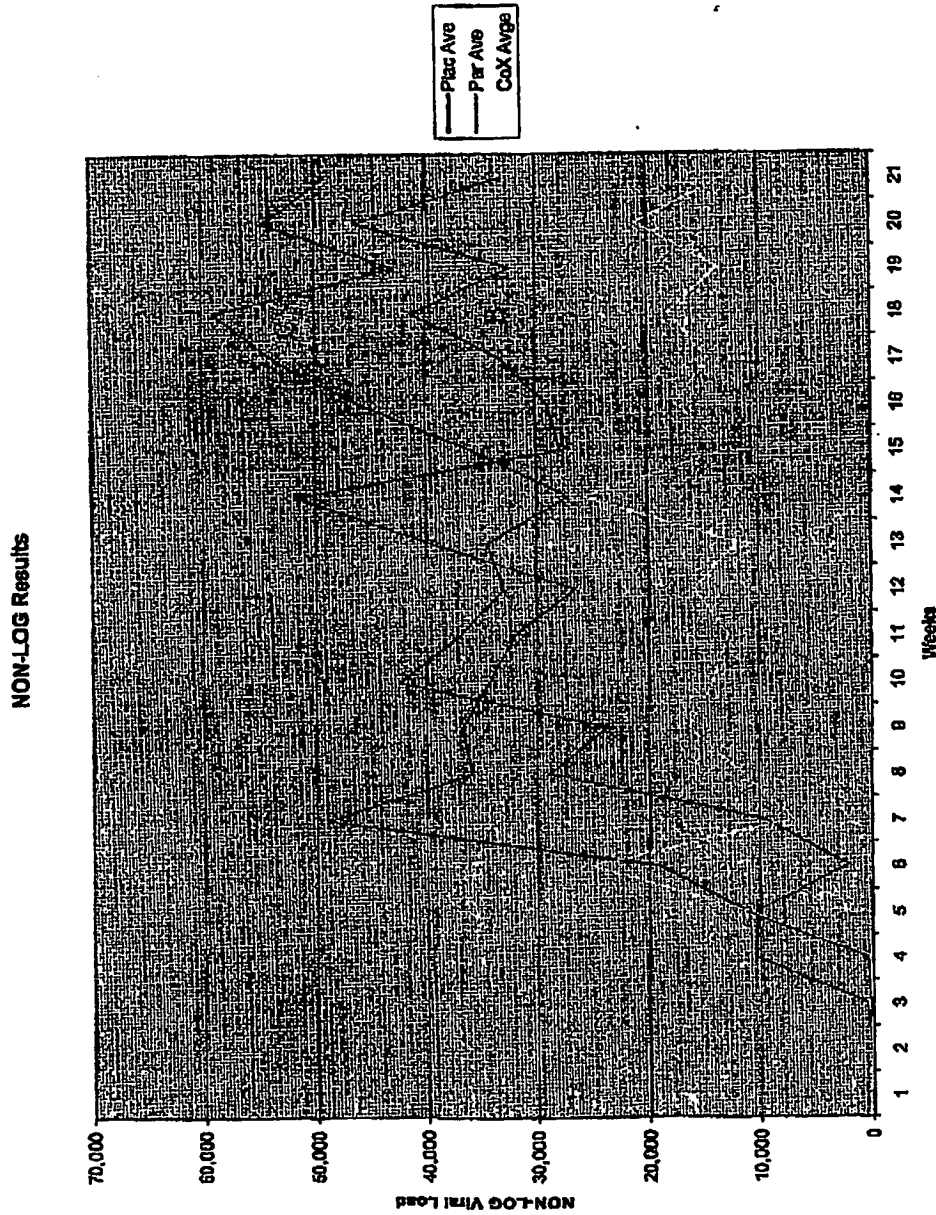


Figure 1

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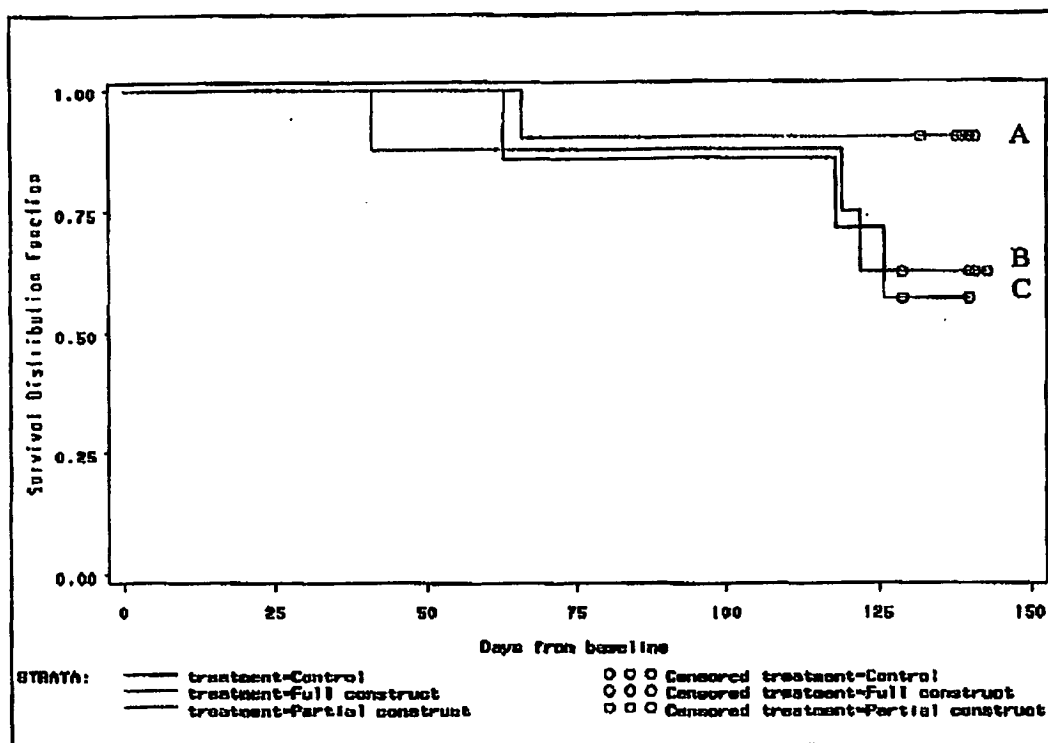


FIGURE 2

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DNA sequence of the insertion site of VIR201 containing HIV gag/pol, human interferon and reporter cassette (Ecogpt & beta-galactosidase) inserts

1 AGACAGTTATCCCAATACGGTATACAAGGAGACAATTATCAATTTTGTAGATTCTTCC
TCTGTCAATAGGGTTATGCCATATGTTCTCTGTAAATAGTTAAAAACATCTAAGAAGG

Fowlpox virus 5' flanking region of insertion site -->

61 AATGAAGTTGCTATAAACAGGCACCTCTATTATAGGAGCTAGACAGTTGAATCCTATATGC
TTACTTCAACGATATTGTCCGTGAGATAATATCCTCGATCTGTCAACTTAGGATATACG

121 GTAGTATCTTTTATCCCTTTTGATCCAGAACATAAAGTTTTTTCGTTATATATGTTGGT
CATCATAGAAAAATAGGGAACCTAGGTCTTGTATTTCAAAAAAGCAATATATACAACCA

181 AGATATAAAGATAAGTATTGTGGAATTTCCTACGTAGCTGATAGAGAAGATATGTACAAA
TCTATATTCTATTCTATAACACACCTTAAAGGATGCATCGACTATCTCTTCTATACATGTTT

241 GTTATCAACAGGATATACCCGTACGTTAGTTGTTTTCCTCGTATCAGATGGTATAATA
CAATAGTTGTCCTATATGGGCATGCAATCAACAATAATGGAGCATAGTCTACCATATTAT

301 AATTTTCATACTACCCGTAGCTAATCACACTAGAAATATTAAACCCCTTCCAGTTAAT
TTAAAAGTATGATGAGGGCATCGATTAGTGTGATCTTTTATAATTTGGGAAGGTCAATTA

FIGURE 3

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361 TATTGTAATACTTTATGTGAAATAGTATATGATTTTGAATATTTAAAGTTTGAACAAGGT
ATAACATTATGAATAACACTTTTATCATATACTAAAACTTATAAATTTCAAACCTTGTTCCA
421 GTTATGTCTATTCCGGTGTTCATGCCCTTTTGTACCAAAACAGTTTGTATCTATTATCAAT
CAATACAGATAAGGCCACAAGTACGGAAACATGGTTTGTGTCAAACATAGATAATAGTTA
481 TTACCAGATGATATTCTCATAAACATGTACAGCGTCCAGTAAACATAGAATACATAACACAT
AATGGTCTACTATAAGAGTATTGTACATGTGCGAGGTCATTGTATCTTATGTATTGTGTA
541 ATAGATAATAAAAGCTAAAGAAATACTTATAATAATAAAGATAAAATTTCTAAAGGGT
TATCTATTATTTTTCGATTTTCTTATGAATATTATTTCTATTTTAAAGATTTTCCCA
601 ACTATCATGCAAGGTACTTTTAAAAAAGTAAATATCATAGACACAAGAGTATACATAT
TGATAGTACGTTCCATGAATAATTTTTCATTTATAGTATTCGTGTCTTCATATGTATA
661 ACTATAACGTATTCTTTTGTATGCCCCTAAACTAGAAAGATACTAAGTCATCGCTGCCA
TGATATTGCATAAGAAGAAACTAACGGGATTTGATCTTCTATGATTCAGTAGCGACGGT
721 AGTACGTGCAATAAAGCCATATTAGATGGGCGTAGATAATGTACAAAAACCTTTTAAATGAT
TCATGCCACGTTATTTCCGGTATAATCTACCCGCATCTATACATGTGTTTTTGAATAATTACTA

FIGURE 3 - CONTINUED

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781 ACAATATAAATGGAAATAGCTAGAGAAACGCTAATAACGATAGGCCTTACTATATTAAGTA
    TGTATATTTACCTTTATCGATCTCTTTGCGATTATTGCTATCCGGAATGATATAATCAT

841 GTGTTATTGATAATAACTGGATTCTCGCTAGTGCTAAGATTAAATACCGGGTGTATTATAGT
    CACAATAACTATTATTGACCTAAGAGCGGATCAGGATTCTTAATTATGGCCCAACAATATCA

901 TCAGTATCGAGGTCAATTTACAGCAGGAAGAAATACTTCGTTTATGGAAATATTTTCT
    AGTCATAGCTCCAGTAGTAAATGTGTCCTCTTCTTATGAAGCAAAATACCTTTATAAAAGA

961 ACTATTATGTTTATTCCTGGATAAATTATATTGTACGCTGCTTATATAAGAAAATTAAT
    TGATAATACAAATAAGGACCTTATTATAATAACATCGCAGCAATATATCTTTTAAATTT

1021 ATGAAAAAATAATTAGAAATCTGAAAATGTCTTCTGGAGCATCCATGTTATTACAGGCCCT
    TACTTTTATTAACTTAGACTTTTACAGAAAGACCTTCGTAGGTACAATAATGTCCGGGA
    > M S S G S I H V I T G P
        Fowlpox virus tk protein coding
sequence →

1081 ATGTTTTCGGTAAACATCGGAGCTAGTAAGAAGATAAAAAGATTATGCTATCTAAC
    TACAAAAGGCCATTTTGAGCCCTCGATCTTCTTATTATTTTCTAAATACGATAGATTG
    > M F S G K T S E L V R R I K R F M L S N

```

FIGURE 3 - CONTINUED

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1141 TTAAATGTATTATTATTAAACATTGTGGAGATAATAGATATATAGGATGATATAAC
AAATTTACATAATAATAATTTGTAAACACCTCTATTATCTATATATTACTCCTACTATATTG
> F K C I I I K H C G D N R Y N E D D I N

1201 AAAGTATATACTCATGATCTATTGTTTATGGAGGCTACGGCATCTTCTAATCTATCTGTA
TTTCATATATGAGTACTAGATAAACAATACTCCGATGCCGTAGAACGATTAGATAGACAT
> K V Y T H D L L F M E A T A S S N L S V

1261 TTAGTACCTACGCTATTAAATGATGGAGTTCAGGTAATAGGTATAGACGAGGCTCAATTC
AATCATGGATGCGATAATTTACTACCTCAAGTCCATTATCCATATCTGCTCCGAGTTAAG
> L V P T L L N D G V Q V I G I D E A Q F

1321 TTTCTAGACATAGTAGAATTTAGTGAATCCATGGCTAATTTAGGTAAACAGTTATTGTG
AAAGATCTGTATCATCTTAAATCACTTAGGTACCGATTAAATCCATTTTGTCAATAACAC
> F L D I V E F S E S M A N L G K T V I V

1381 GCCGCGCTTAACGGTGATTTTAAACGCGAATTATTTCGGTAAACGTATATAAGTTATTATCA
CGGCGCGAATTGCCCACTAAATTTGCGCTTAATAAGCCATTGCATATATTCAATAATAGT
> A A L N G D F K R E L F G N V Y K L L S

FIGURE 3 - CONTINUED

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1441 TTAGCTGAACAGTGTCAGTTCAGACAGCTATTGGGTGAAATGCTATTGCGACGCTTCG
    AATCGACTTTGTGCACAGGTCAAACTGTGCGATAAACGCACTTTACGATAAACGCTGCGAAGC
    > L A E T V S S L T A I C V K C Y C D A S

1501 TTTTCTAAACGAGTTACAGAAATAAAGAAGTAATGGATATAGGTGGTAAAGATAAATAC
    AAAAGATTGCTCAATGCTCTTTTATTCTTCATTACCTATATCCACCACTTCTATTATG
    > F S K R V T E N K E V M D I G G K D K Y

1561 ATAGCCGTGTAGGAAATGTTTTTTTAGTAATTAAAGGGGAGATctcccatggcccaa
    TATCGGCACACATCCTTTACAAAAAATCATTAATTccccctctagaggggtaccggggttt
    > I A V C R K C F F S N •

1621 gcggggttgaacagggttcgctcaggtttgcctgtgtcatggatgcagcctccagaat
    cgcccaacttgtccaaagcaggtccaaacggacacagtcactacgtcggaggtctta

1681 acttactgaaactattgtaaccgcctgaagttaaaagaacaacgcccgagtgcca
    tgaatgaccttggataacattggcggaacttcaattttctgttgcgggcccgtcacggt

1741 ggcgttgaaaaaattAGCGACCGGAGATTGGCGGACGAATACGACGCCCATATCCACG
    ccgcaacttttctAATCGCTGGCCTCTAACCGCCCTGCTTATGCTGCGGGTATAGGTGC
    < • R G S I P P V F V G M D W P

```

FIGURE 3 - CONTINUED

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End of Ecogpt protein coding sequence

1801 GCTGTTCAATCCAGGTATCTTGGGGATATCAACAATAGTCAACACGCGGACGAC
 CGACAAGTTAGGTCCATAGAACGCCCTATAGTTGTGTATCAGTAGTTGGTCGCTGCTG
 < Q E I W T D Q P I D V V Y D D V L P R G

 1861 CAGCCGGTTTTCGGAAGATGGTGACAAAGTGGCTTTTGGATACATTTACGAATCGCAA
 GTCGGCCAAACGCTTCTACCACTGTTCACGCGAATAACCTATGTAAAGTCTTAGCGTT
 < A P K A F I T V F H A K P Y M E R I A V

 1921 CCGCAGTACCAACCGGTATCCACCAGGTCAATCAATAACGATGAAGCCTTCGCCATCGCCTT
 GCGTCAATGGTGGCCATAGGTGTCCAGTAGTTATTGCTACTTCGGAAGCGGTAGCGGAA
 < A T G G T D V L D I V I F G E G D G E

 1981 CTGCGCGTTTCAGCACTTTAAGCTCGCGCTGGTTGTCTGATCGTAGCTGGAATAACAA
 GACGCGCAAGTCTGTGAAATTCGAGCGCGACCAACAGCACTAGCATCGACCTTTATGTTT
 < A R K L V K L E R Q N D H D Y S S I C V

 2041 CGGTATCGACATGACGAATACCCAGTTCAACGCGCCAGTAACGACCCGGTACCAGACCGC
 GCCATAGCTGTACTGCTTATGGTCAAGTGCGCGGTCAATGCGTGGCCATGGTCTGGCG
 < T D V H R I G L E R A L L A G P V L G G

FIGURE 3 - CONTINUED

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2101 CACGGCTTACGGCAATAATGCCCTTTCATTTGTTCAAGGCAATAGTCGGCTTGGAGTT
 GTGCCGAATGCCGTTATTACGGAAGGTAACAAGTCTTCCGTAGTCAGCCGAAACGCTCAA
 < R S V A I I G K W Q E S P M L R S A L K

2161 TACGTGCATGGATCTGCAACATGTGCCAGGTGACGATGTAATTTTCGCTCATgtgaagtg
 ATGCACGTACCTAGACGTTGTACAGGGTCCACTGCTACATAAAAGCGAGTAcacttcac
 < R A H I Q L M D W T V I Y K E S M
 ←start of *Ecogpt* protein coding sequence

2221 tcccagcctgtttatctacggcttaaaagtgttcgaggggaaaaataggttgcgcgagat
 agggtcggacaaatagatgccgaattttcacaaagctccccttttatccaacgcgctcta

2281 tatagagatccgtcactgttctttatgatctacttccttaCCGTGCAATAAATTAGAATA
 atatctctaggcagtgacaagaataactagatgaaggaatGGCACGTTATTTAATCTTAT

2341 TATTTTCTACTTTTACGAGAAATTAATTATTGTAATTTATTATGCGTGAAAAACTTA
 ATAAAAGATGAAAATGCTCTTTAATTAATAACATAATAATAATACCCACTTTTGAAT
 ← *Vaccinia virus p7.5* promoter (marked in upper case)

2401 CTATAAAAAGCGGGTGGGTTTGGAattagtgatcagtttatgtatatcgcaactaccggc
 GATATTTTTCGCCCCACCCAAACCTtaactagtcataatacatatagcgttgatggccg

FIGURE 3 - CONTINUED

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2461 atatggctattcgacatcgagaacattaccacatgataagagattgtatcagtttcgta
 tataccgataagctgtagctcttgtaatgggtgtactattcttaacatagtc aaagcat

2521 gtcttgagtattggtattactatatagtatatgtcgggaattcagatccatgcagatccc
 cagaactcataaccataatgatataatcatatacagcccttaagtctaggctacgtcttaggg

2581 ccctgccccggttattaTTATTTTGACACCAGACCAACTGGTAATGGTAGCGACCGCGC
 gggacgggccaataataATAAAACTGTGGTCTGGTTGACCATTAACATCGCTGGCCGCG

< • K Q C W V L Q Y H Y R G A S
 End of beta-Galactosidase protein coding

sequence

2641 TCAGCTGGAATTCGCCGATACACTACGGGCTCCAGGAGTCGTCGCCACCAATCCCCATAT
 AGTCGACCTTAAGCGGCTATGACTGCCCGAGGTCTCAGCAGCGGTGTTAGGGGTATA
 < L Q F E A S V S P S W S D D G G I G M H

2701 GGAAACCGTCGATATTCAGCCATGTGCCCTTCTTCCGCGTGCGAGCAGATGGCGATGGCTGG
 CCTTTGGCAGCTATAAGTCGGTACACGGAAGAGCGCACGTCGCTACCGCTACCGACC
 < F G D I N L W T G E E A H L L H R H S T

FIGURE 3 - CONTINUED

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2761 TTTCCATCAGTTGCTGTGACTGTAGCGGTGATGTTGAACCTGGAAGTCGCCGCGCCACT
AAAGGTAGTCAACGACAACTGACATCGCCGACTACAACCTTGACCTTCAGCGCGCGGTGA
< E M L Q Q Q S Y R S I N F Q F D G R W Q

2821 GGTGTGGCCATAATTCAATTCGCGCTCCCGCAGCGCAGACCGTTTTCGCTCGGGAAGA
CCACACCCCGTATTAGTTAAGCGCGCAGGCGTCCGCTCTGGCAAAAGCAGCCCTTCT
< H P G Y N L E R T G C R L G N E S P F V

2881 CGTACGGGGTATACATGCTGACAAATGGCAGATCCCAGCGGTCAAACAGCGGCAGTAA
GCATGCCCCCATATGTACAGACTGTACCGTCTAGGTCGCCAGTTTGTCCGCCGTCAAT
< Y P T Y M D S L P L D W R D F C A A T L

2941 GCGGTCGGGATAGTTTCTTGGGCCCTAATCCGAGCCAGTTTACCCGCTCTGCTACCT
CCGCCAGCCCTATCAAAGAACGCCGGGATTAGGCTCGGTCAAATGGCGGAGACGATGGA
< R D P Y N E Q P G L G L W N V R E A V Q

3001 GCGCCAGCTGGCAGTTCAGGCCAATCCGCCCGGATGCGGTGTATCGCTCGCCACTTCAA
CGCGGTGACCGTCAAGTCCGGTTAGGCGCGGCCCTACGCCACATAGCGAGCGGTGAAGTT
< A L Q C N L G I R A P H P T D S A V E V

3061 CATCAACGGTAATCGCCATTGTACCACCTACCATCAATCCGGTAGGTTTCCGGCTGATAA

FIGURE 3 - CONTINUED

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GTAGTTGCCATTAGCGGTAACTGGTGATGGTAGTTAGGCCATCCAAAGGCCGACTATT
 < D V T I A M Q G S G D I R Y T K R S I F

 3121 ATAAGGTTTCCCTGATGCTGCCACGCGTGAGCGGTGTAATCAGCACCGCATCAGCAA
 TATTCCAAAAGGGACTACGACGGTGGCACTCGCCAGCATTAGTCGTGGCGTAGTCGTT
 < L T K G Q H Q W A H A T T I L V A D A L

 3181 GTGTATCTGCGTGCACTGCAACACGCTGCTTCGGCCTGGTAATGGCCCCGCCCTTCC
 CACATAGACGGCACGTGACGTTGTTCGACGAGAACCGGACCATTACCGGGCGGGAAGG
 < T D A T C Q L L A A E A Q Y H G A A K W

 3241 AGCGTTCGACCCAGCGTTAGGGTCAATGCGGGTCGCTTCACCTACGCCAATGTCGTAT
 TCGCAAGCTGGTCCGCAATCCAGTTACGCCCCAGCGAAGTGAATGCGTTACAGCAATA
 < R E V W A N P D I R T A E S V G I D N D

 3301 CCAGCGGTGCACGGGTGAACCTGATCCGCGCAGCGCGTCAGCAGTTGTTTATCGCCAA
 GGTCCGCCACGTGCCCACTTGACTAGCGCGTCGCGCAGTCGTCAACAAATAAGCGGTT
 < L P A R T F Q D R L P T L L Q K K D G I

 3361 TCCACATCTGTGAAGAAAGCCTGACTGGCGGTTAAATTGCCAACGCTTATACCCAGCT
 AGGTGTAGACACTTCTTTCGGACTGACCGCCCAATTAAACGGTTGCGAATAATGGGTGGA

FIGURE 3 - CONTINUED

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< W M Q S L F G S Q R N F Q W R K N G L E
 3421 CGATGCAAAATCCATTTCGCTGGTGCAGATCGGGATGGCGTGGACGCGGGGGGA
 GCTACGTTTTAGGTAAAGCGACCAAGTCTACGCCCTACCGCACCTGCGCGCCCT
 < I C F D M E S T T L H P I A H S A A P L
 3481 GCGTCACACTGAGGTTTCCGCCAGACGCCACTGCTGCCAGGCGCTGATGTGCCCCGGCTT
 CGCAGTGTGACTCCAAAGCGGCTCTGCGGTGACGACGCTCCGCGACTACACGGGCCGAA
 < T V S L N E A L R W Q Q W A S I H G A E
 3541 CTGACCATGCGGTGCGTTCGGTTGCACCTACGCTACTGTGAGCCAGAGTTGCCCGGCGC
 GACTGCTACGCCAGCGCAAGCAACGTCGTCATGCGTCACTCGGTCTCAACGGGCCGCG
 < S W A T A N P Q V V R V T L W L Q G A S
 3601 TCTCCGGCTGCGGTAGTTCAGGCAGTTCATCAACTGTTTACCTTGTGGAGCGCATCCA
 AGAGGCCGACGCCATCAAGTCCGTCAAGTTAGTTGACAAATGGAACACCTCGCTGTAGT
 < E P Q P L E P L E I L Q K G Q P A V D L
 3661 GAGGCACTTACCGCTTGCCAGCGGCTTACCATCCAGCGCCACCATCCAGTGCAGGAGCT
 CTCCGTGAAGTGGCGAACGTCGCCGAATGGTAGTTCGCGGTGGTAGTCACTCTCGA
 < P V E G S A L P K G D L A V M W H L L E

FIGURE 3 - CONTINUED

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3721 CGTTATCGCTATGACGGAACAGGTATTCCGCTGGTCACCTTCGATGGTTGCCGCGATAAAC
GCAATAGCGATACTGCCCTTGTCCTAAGCGACCAGTGAAGCTACCAACCGGCCCTATTG
< N D S H R F L Y E S T V E I T Q G S L R

3781 GGAACTGGAAAACTGCTGCTGGTGTTTGGCTTCGGTCAGCGCTGGATCGCGGTGCGGT
CCTTGACCTTTTGTGACGACGACCACAAAACGAAGCAGTCGCGACCTACGCCGACGCCA
< F Q F F Q Q Q H K A E T L A P H P T R D

3841 CGGCAAAGACCAGACCGTTTCATACAGAACTGGCGATCGTTCCGGGTATCGCCAAAATCAC
GCCGTTTCTGGTCTGGCAAGTATGCTTGACCGCTAGCAAGCCGCATAGCGGTTTGTAGTG
< A F V L G N M C F Q R D N P T D G F D G

3901 CGCCGTAAGCCGACCACGGGTGCGGTTTTCATCATATTAAATCAGCGACTGATCCACCC
GCGGCATTGCGCTGGTGCCCAACGGCAAAAGTAGTATAAATTAGTCGCTGACTAGGTGGG
< G Y A S W P N G N E D Y K I L S Q D V W

3961 AGTCCCAGACGAAGCCGCCCTGTAAACGGGGATACTGACGAAACGCCCTGCCAGTATTAG
TCAGGGTCTGCTTCGGCGGGACATTTGCCCTATGACTGCTTTGCGGACGGTCATAAATC
< D W V F G G Q L R P Y Q R F A Q W Y K A

FIGURE 3 - CONTINUED

15/38

4021 CGAAACCGCCAAGACTGTTACCCATCGCGTGGCGGTATTCGCAAGGATCAGCGGCGCG
GCTTTGGCGGTTCTGACAAATGGGTAGCGCACCCGCATAAGCGTTTCTAGTCCCGCGCGC
< F G G L S N G M A H A Y E C L I L P R T

4081 TCTCTCAGGTAGCGAAGCCATTTTGTGATGGACCATTTTCGGCACAGCCGGGAAGGCT
AGAGAGGTCATCGCTTTCGGTAAATAAACTACCTGGTAAAGCCGTGTGGCCCTTCCCGA
< E G P L S L W K K I S W K P V A P F P Q

4141 GGTCTTCATCCACGCGCGGTACATCGGGCAATAATATCGGTGGCCGTGGTGTGGCTC
CCAGAAGTAGGTGCGCGCATGTAGCCCGTTTATTATAGCCACCGGCACACAGCCGAG
< D E D V R A Y M P C I I D T A T T D A G

4201 CGCCGCCCTTCATACTGCACCGGGCGGAAGGATCGACAGATTGTATCCAGCGATACAGCG
GCGGCGGAAGTATGACGTGGCGCCGCCCTTCCCTAGCTGTCTAAACTAGGTGGCTATGTGCG
< G G E Y Q V P R S P D V S K I W R Y L A

4261 CGTCGTGATTAGCGCCGTGGCCTGATTTCATTCGCCAGCGACAGATGATCACATCGGGT
GCAGCACTAATCGCGGCACCGGACTAAGTAAGGGTGGTGGTCTACTAGTGTAGCCCCA
< D H N A G H G S E N G L S W I I V S P H

4321 GATTACGATCGCGCTGCACCATTCGCGTTACGCGTTGCTCATCGCCGGTAGCCAGCGCG

FIGURE 3 - CONTINUED

16/38

CTAATGCTAGCGGACGTGGTAAGCGCAATGCGCAAGCGAGTAGCGGCCATCGGTGCGGC
< N R D R Q V M R T V R E S M A P L W R P
4381 GATCATCGGTCAGACGATTTCATTGGCACCATGCCGTGGGTTTCAATATTGGCTTCATCCA
CTAGTAGCCAGTCTGCTAAGTAAACCGTGGTACGGCACCCAAAGTTATAACCGAAGTAGGT
< D D T L R N M P V M G H T E I N A E D V
4441 CCACATACAGGCCGTAGCGGTCCACAGCGGTGTACACAGCGGATGGTTCGGATAATGCCG
GGTGTATGTCCGGCATCGCCAGCGGTGTGCGCACATGGTGTGCGCTACCAAGCCTATTACGC
< V Y L G Y R D C L T Y W L P H N P Y H S
4501 AACAGCGCACGGGTTAAAGTTGTCTGCTTCATCAGCAGGATATCCTGCACCATCGTCT
TTGTCCGGTGCCGCAATTTCACAACAGACGAAGTAGTCTGTCCTATAGGACGTGGTAGCAGA
< C R V A N F N N Q K M L L I D Q V M T Q
4561 GCTCATCCATGACCTGACCATGCAGAGGATGATGCTCGTGACGGTTAACGCCCTCGAATCA
CGAGTAGTACTGGACTGGTACGTCTCCTACTACGAGCACTGCCAATTGCGGAGCTTAGT
< E D M V Q G H L P H H E H R N V G R I L
4621 GCAACGGCTTGCCGTTACAGCAGCAGACCAATTTTCAATCCGCACCTCGCGGAAACCGA
CGTTGCCGAACGGCAAGTCGTCTGTTGGTAAAGTTAGCGGTGGAGCGCCTTTGGCT

FIGURE 3 - CONTINUED

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4681 CATCGCAGGCTTCTGCTTCAATCAGCGTCCCGTGGCGGTGTGCACTTCAACCAACCGCAC
GTAGCGTCCGAAGACGAAGTTAGTCGACGGCAGCCGCACACGTCAAGTTGGTGGCGTG
< D C A E A E I L T G D A T H L E V V A R

4741 GATAGAGATTCGGGATTTCCGGCTCCACAGTTTCGGGTTTTCGACGTTCAGACGTAAGT
CTATCTCTAAGCCCTAAAGCCGCGAGGTGTCAAGCCCAAAGCTGCAAGTCTGCATCAC
< Y L N P I E A S W L K P N E V N L R L T

4801 TGACGCGATCGGCATAACCAACCGCTCATCGATAATTTCACCGCCGAAAGGCGCGGTGC
ACTGCGCTAGCCGTATTGGTGTGCGAGTAGCTATTAAAGTGGCGGCTTTCGCGGCCACG
< V R D A Y G G R E D I I E G G F P A T G

4861 CGCTGGCGACCTGCGTTTCACCCCTGCCATAAAGAAACTGTTCACCCGTAGTAGTCACGCA
GGACCGCTGGACGCAAGTGGGACGGTATTTCTTTTGACAATGGGCATCCATCAGTCCGT
< S A V Q T E G Q W L S V T V R L Y D R L

4921 ACTCGCCGCACATCTGAACCTTCAGCCTCCAGTACAGCGCGGCTGAAATCATCATTAAGC
TGAGCGGCGGTGTAGACTTGAAGTCGGAGGTCACTGTCGCGCCGACTTTAGTAGTAATTTCG
< E G C M Q V E A E L V A R S F D D N F R

FIGURE 3 - CONTINUED

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4981 GAGTGGCAACATGGAAATCGCTGATTGTGTAGTCGGTTTATGCAGCAACGAGACGTCAC
CTCACCCGTTGTACCTTTAGCGACTAAACACATCAGCCAAATACGTCGTTGCTCTGCAGTG
< T A V H F D S I Q T T P K H L L S V D R

5041 GGAAATGCCGCTCATCCGCCACATATCCTGATCTTCCAGATAACTGCCGTCACCTCCAAC
CCTTTTACGGCGAGTAGGCGGTGTATAGGACTAGAGGTCTATTGACGGCAGTGAGGTTG
< F I G S M R W M D Q D E L Y S G D S W R

5101 GCAGCACCATCACCGCGAGGCGGTTTCTCCGGCGGTAAATAATGCGCTCAGGTCAAATT
CGTCGTGGTAGTGGCGCTCCGCCAAAGAGGCCGCGCATTTTACGCGAGTCCAGTTTAA
< L V M V A L R N E G A R L F A S L D F E

5161 CAGACGGCAACGACTGTCTGGCCGTAACCGACCCAGCGCCCGTTGCACACAGATGAA
GTCTGCCGTTTGTCTGACAGGACCGGCATTGGCTGGTCGCGGGCAACGTGGTGTCTACTT
< S P L R S D Q G Y G V W R G N C W L H F

5221 ACGCCGAGTTAACGCCCATCAAAAATAATTGCGTCTGGCCTTCTGTAGCCAGCTTTCAT
TGCGGCTCAATTGCGGTAGTTTATTATTAAGCGCAGACCGGAAGGACATCGGTCGAAAGTA
< A S N V G D F I I R T Q G E Q L W S E D

5281 CAACATTAAATGTGAGCGAGTAACAACCCGTCGGATTCTCCGTGGGAACAACGCGCGAT

FIGURE 3 - CONTINUED

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GTTGTAAATTACACTCGCTCATTTGTTGGGCAGCCTAAGAGGCACCCTTGTTCGCCGCTA
 < V N F T L S Y C G T P N E T P V F P P N

 5341 TGACCGTAATGGGATAGGTTACGTTGGTGTAGATGGCGCATCGTAACCGTGCACTGCCC
 ACTGGCATTAACCCATATCCAATGCAACACATCTACCCGCGTAGCATTTGGCACGTAGACGG
 < V T I P Y T V N T Y I P A D Y G H M Q W

 5401 AGTTTGAGGGACGACAGTATCGGCCCTCAGGAAGATCGCACTCCAGCCAGCTTTCGG
 TCAAACTCCCTGCTGCTGTCTATAGCCGAGTCTTCTAGCGTGAGGTGCGTCGAAAGGC
 < N S P V V T D A E P L D C E L W S E P

 5461 GCACCGCTTCTGTGCGGAAACAGGCAAGCGCCATTCCGCATTAGGCTGCGCAACT
 CGTGGCGAAGACCAACGCGCTTTGTCCGTTTCGGGTAAGCGGTAAGTCCGACGCGTTGA
 < V A E P A P F W A F R W E G N L S R L Q

 5521 GTTGGGAAGGCGATCGGTGCGGCCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGAT
 CAACCCCTTCCCGTAGCCACGCCCGGAGAGCGATAATGCGGTGACCGCTTTCGCCCTTA
 < Q S P R D T R A E E S N R W S A F P P H

 5581 GTGCTGCAAGCGATTAGTTGGTAACGCCAGGTTTTCAGTCACGACGTTGTAAAA
 CACGACGTTCCGCTAATTCAACCCATTGCGGTCCCAAAGGTCAGTGTGCAACATTTT

FIGURE 3 - CONTINUED

20/38

< A A L R N L Q T V G P N E W D R R Q L V
 5641 CGACGGGATCTAGCATggatctagccatttagtattcctaataattgaattgtaattatcgga
 GCTGCCCTAGATCGTACctagatcggtaaatcataggaattttaacttaacattaatagctt
 < V P D L M
 ←start of beta-Galactosidase protein coding
 sequence
 ← Fowlpox virus bidirectional promoter (in
 bold) →
 5701 **TAATAAATGgacggatcgATGAAATATACAAGTTATATCTTGGCTTTTCAGCTCTGCATC**
ATTATTFACctgcctagctACTTTATATGTTCAATATAGAACCGAAAGTCGAGACGTAG
 > M K Y T S Y I L A F Q L C I
 Human interferon gamma protein coding
 sequence →
 5761 GTTTGGGTTCTCTTGGCTGTTACTGCCAGGACCCATATGTAAAGAGCAGAAACCTT
 CAAACCCAGAGAACCGACAATGACGGTCTGGGTATACATTTCCTTCGTCCTTTTGGA
 > V L G S L G C Y C Q D P Y V K E A E N L
 5821 AAGAAATATTTTAATGCAGGTCATTcAGATGTAGCGGATAATGGAACTCTTTTCTTAGGC

FIGURE 3 - CONTINUED

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TTCTTTATAAAATTACGTCAGTAAGTCTACATCGCCTATTACCTTGAGAAAAGAATCCG
> K K Y F N A G H S D V A D N G T L F L G

5881 ATTTTGAAGAAATTGGAAAGAGGAGAGTGACAGAAATAATGCAGAGCCAAATTGTCTCC
TAAACTTCTTAACCTTTCTCCTCTCCTCCTCCTGTTCTTTTATTACGTCTCGGTTTAAACAGAGG
> I L K N W K E E S D R K I M Q S Q I V S

5941 TTTTACTTCAAACCTTTTAAAAAACCTTTAAAGATGACCAGAGCATCCAAAAGAGTGTGGAG
AAAATGAAGTTTGAAAAAATTTTGAATAATTTCTACTGGTCTCGTAGGTTTCTCACACCTC
> F Y F K L F K N F K D D Q S I Q K S V E

6001 ACCATCAAGGAAGACATGAATGTCAAGTTTTCATAATAGCAACAAAAGAACGAGATGAC
TGGTAGTTCCCTTCTGTACTTTACAGTTCAAAAAGTTATCGTTGTTTTCCTTGTCTCTACTG
> T I K E D M N V K F F N S N K K R D D

6061 TTCGAAAAGCTGACTAATTAATTCGGTAAGTGAATGTCCAACGCAAGCAATACAT
AAGCTTTTCGACTGATTATAAGCCATTGACTGAAGTTACAGGTTGCGTTTCGTTATGTA
> F E K L T N Y S V T D L N V Q R K A I H

6121 GAACTCATCCAAGTGATGGCTGAAGTGTGCCAGCAGCTAAAACAGGGAAGCGAAAAGG
CTTGAGTAGGTTCACTACCGACTTGACAGCGGTGTCGATTTTGTGCCCTTCGCTTTTCC

FIGURE 3 - CONTINUED

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> E L I Q V M A E L S P A A K T G K R K R
6181 AGTCAGATGCTGTTTCGAGGTCGAAGAGCATCCAGTAATggttgctcctgccaatat
TCAGTCTACGACAAAGCTCCAGCTTCTCGTAGGGTCATTaccaacaggacggacggttata
> S Q M L F R G R A S Q •
6241 ttgaattttaaatctaattattttaataatttaacattatttatatggggaatatat
aacttaaaatttagatttagataaaataattataaaattgtaataatatatacccttatata
6301 ttttagactcatcaatcaataagttattataatagcaactTTTTGtaatggatccc
aaaatctgagtagtagttatttcataaataattatcggttgAAAAACattacctaggg
Engineered transcriptional stop motif (in
upper case)
6359 agctctctcgacgaggactcggcttgctgaagcgcgcacagcaagagggcgagggcggc
tcgagagagctgcgtcctgagccgaacgacttcgcgcgtgtcgttctccgctccccgcgcg
6419 gactggtgagtacgccaatttttgactagcggaggctagaaggagagagagATGGGTGCG
ctgaccactcatgcggttaaaaaactgatcgccctccgatcttcctctctctTACCCACGC
> M G A
HIV gag protein coding

```

FIGURE 3 - CONTINUED

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sequence →

```
6479 AGAGCGTCGGTATTAGCGGGGAGAAATTAGATAAATGGGAAAAAATTCGGTTAAGGCCA
    TCTCGCAGCCATAATTGCCCCCTCTTAATCTATTACCCCTTTTTAAGCCAATTCCGGT
    > R A S V L S G G E L D K W E K I R L R P

6539 GGGGAAAGAAAAATATAAGTTAAACATATAGTATGGCAAGCAGGGAGCTAGAACGA
    CCCCCTTTCTTTTATATATTCAATTTTGTTATATCATACCCGTTGTCCTCGATCTTGCT
    > G G K K Y K L K H I V W A S R E L E R

6599 TTCGCAGTCAATCCTGGCCTGTTAGAAACATCAGAAGGCTGCAGACAAATATGGGACAG
    AAGCGTCAGTTAGGACCGGACAAATCTTTGTAGTCTTCCGACGTCGTGTTATAACCCGTGC
    > F A V N P G L L E T S E G C R Q I L G Q

6659 CTACAGCCATCCCTTCAGACAGGATCAGAAAGAACTTAGATCATTATATAACAGTAGCA
    GATGTCGGTAGGGAAGTCTGTCTCCTAGTCTTCTTGAATCTAGTAAATATATATGTCATCGT
    > L Q P S L Q T G S E E L R S L Y N T V A

6719 ACCCTCTATTGTGTACATCAAGGATAGATGTAAAGACACCAAGGAAGCTTTAGAGAAG
    TGGGAGATAACACATGTAGTTTCTCTATCTACATTTTCTGTGGTTCCCTTCGAAATCTCTTC
    > T L Y C V H Q R I D V K D T K E A L E K
```

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FIGURE 3 - CONTINUED

```

6779 ATAGAGGAAGAGCAAAACAAAAGTAAGAAAAGGCACAGCAAGCAGCAGCTGCAGCTGGC
    TATCTCCTTCTCTCGTTTGTGTTTTCATTCTTTTCCGTGTCGTTCGTGACGACCG
    > I E E E Q N K S K K A Q Q A A A A A G

6839 ACAGGAAACAGCAGCCAGGTCAGCCAGCAAAATTACCTATAGTGCAGAACCTACAGGGGCAA
    TGTCCCTTTGTGTCGTCCAGTCGGTTTAAATGGGATATCACGTCCTTGGATGTCCCGTT
    > T G N S S Q V S Q N Y P I V Q N L Q G Q

6899 ATGGTACATCAGGCCATATCACCTAGAACCTTAAATGCATGGGTAAAGTAGTAGAAGAA
    TACCATGTAGTCGGTATAGTGGATCTTGAAATTACGTACCCATTTCATCATCTTCTT
    > M V H Q A I S P R T L N A W V K V V E E

6959 AAGGCTTTCAGCCCAGAAAGTAATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCA
    TTCCGAAAGTCGGGTCTTTCATTATGGGTACAAAGTCGTAATAGTCTTCCTCGGTGGGT
    > K A F S P E V I P M F S A L S E G A T P

7019 CAAGATTTAACACCATGCTAAACACAGTGGGGGACATCAAGCAGCCCATGCAATGTTA
    GTTCTAAATTGTGTGACGATTGTGTCAACCCCTGTAGTTCGTGCGTACGTTTACAAT
    > Q D L N T M L N T V G G H Q A A M Q M L

```

FIGURE 3 - CONTINUED

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7079 AAAGAGACTATCAATGAGGAAGCTGCAGAAATGGGATAGAGTGCATCCAGTGCATGCAGGG
TTTCTCTGATAGTTACTCCTTCGACGTCCTTACCCCTATCTCACGTAGGTACGTACGTCCC
> K E T I N E E A A E W D R V H P V H A G

7139 CCTATTGCACCAGGCCAAATGAGAGAACCAAGGGGAAGTGACATAGCAGGAACCTACTAGT
GGATAACGTGGTCCGGTTTACTCTCTTGGTTCCCTTCACTGTATCGTCTTGATGATCA
> P I A P G Q M R E P R G S D I A G T T S

7199 ACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCAGTAGGAGAAATC
TGGGAAGTCCTTGTATTATCCTACTACTGTATTATTAGTGGATAGGGTCACTCCTCTTTAG
> T L Q E Q I G W M T N N P P I P V G E I

7259 TATAAAGATGGATAATCCTGGGATTAAATAAATAGTAAGAAATGTATAGCCCTACCAGC
ATATTTTCTACCTATTAGGACCCTAATTATTATTATCATTCTTACATATCGGGATGGTCG
> Y K R W I I L G L N K I V R M Y S P T S

7319 ATTCTGGACATAAGACAAGGACCAAGGAACCTTTAGAGATTATGTAGACCGGTTCTAT
TAAGACCTGTATTCTGTTCCTGGTTTCCCTGGGAAATCTCTAATAACATCTGGCCCAAGATA
> I L D I R Q G P K E P F R D Y V D R F Y

7379 AAAACTCTAAGAGCCGCAACAAGCTTCACAGGATGTAAAAATTGGATGACAGAAACCTTG

FIGURE 3 - CONTINUED

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```

TTTGTGAGATTCTCGGCTTGTTCGAAGTGTCCTACATTTTAAACCTACTGTCTTTGGAAAC
> K T L R A E Q A S Q D V K N W M T E T L

7439 TTGGTCCAAATGCAAAACCAGATTGTAAGACTATTTTAAAGCATTTGGGACCAGCAGCT
AACCAAGTTTACGTTTGGGTCTAACATTCTGATAAAATTTTCGTAACCCCTGGTCGTCGA
> L V Q N A N P D C K T I L K A L G P A A

7499 ACACTAGAAGAAATGATGACAGCATGTGAGGAGTGCGGGGACCCGGCCATAAAGCAAGA
TGTGATCTTCTTTACTACTGTGTCGTACAGTCCCTCACCCCCCTGGGCCGGTATTTTCGTTCT
> T L E E M M T A C Q G V G G P G H K A R

7559 GTTTTGGCTGAAGCCATGAGCCAAAGTAACAAATCCAGCTAACATAATGATGCAGAGAGGC
CAAAACCGACTTCGGTACTCGGTTCATTGTTTAGGTCGATTGTAATTACTACGTCTCTCCG
> V L A E A M S Q V T N P A N I M M Q R G

7619 AATTTAGGAACCAAGAAAGACTGTAAAGTGTTCAAATTGTGGCAAGAAAGGCACATA
TTAAATACTCTTGGTTTCTTCTGACAAATTCACAAAGTTAACACCGTTTCTTCCCGTGTAT
> N F R N Q R K T V K C C F N C G K E G H I

7679 GCCAAAAATTGCAGGGCCCCTAGGAAAGGGCTGTGGAGATGTGGAAGGGAAGGACAC
CGGTTTAAACGTCCTCCGGGATCCTTTTCCCGACAAACCTCTACACCTTCCCTTCCCTGTG

```

FIGURE 3 - CONTINUED

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> A K N C R A P R K K G C W R C G R E G H
 7739 CAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTATAGGAAGATCTGGCCTTCCTAC
 GTTACTTTCTAACGTGACTCTGTCCGATATAAAATCCCTTCTAGACCGGAAGGATG
 > Q M K D C T E R Q A N F L G K I W P S Y
 > F F R E D L A F L
 HIV pol protein coding
 sequence →

7799 AAGGGAAGCCAGGGAATTTCTTCAGAGCAGACCAGAGCCCAACAGCCCCACCAGAAGAG
 TTCCCTTCCGGTCCCTTAAAGAAGTCTCGTCTGGTCTCGGTTGTGGGTGCTTCTC
 > K G R P G N F L Q S R P E P T A P P E E
 > Q G K A R E F S S E Q T R A N S P T R R

7859 AGCTTCAGGTTTGGGGAGGAGAAACAACCTCCTCTCAGAAGCAGGAGCCGATAGACAAG
 TCGAAGTCCAAACCCCTCCTCTTTTGTGAGGGAGAGTCTTCGTCTCGGCTATCTGTTC
 > S F R F G E E K T T P S Q K Q E P I D K
 > E L Q V W G G E N N S L S E A G A D R Q

7919 GAACGTATCCTTTAACTTCCTCAGATCACTTTGGCAACGACCCCTCGTCACAATAA
 CTTGACATAGGAAATTGAAGGGAGTCTAGTGAGAAACCGTTGCTGGGAGCAGTGTATT

FIGURE 3 - CONTINUED

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> E L Y P L T S L R S L F G N D P S S Q •
> G T V S F N F P Q I T L W Q R P L V T I

7979 GGATAGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAG
CCTATCCCCCGTTGATTTCCCTTCGAGATAATCTATGTCTCTGTTACTATGTCTATAATC
> R I G G Q L K E A L L D T G A D D T V L

8039 AAGAAATGAATTTGCCAGGAAATAATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTAA
TTCTTTACTTAAACGGTCCCTTTTACCTTTTGGTTTTTACTATCCCCCTTAAACCTCCAAAAT
> E E M N L P G K W K P K M I G G I G G F

8099 TCAAAGTAAGACAGTACGATCAGATACCTGTAGAAAATCTGTGGACATAAAGCTATAGGTA
AGTTTCATTTCTGTCAATGCTAGTCTATGGACATCTTTAGACACCTGTATTTTCGATATCCAT
> I K V R Q Y D Q I P V E I C G H K A I G

8159 CAGTATTAGTAGGACCTACACCTGTCAACATAAATTGGAAAGAAATCTGTGACTCAGATTG
GTCATAATCATCCTGGATGTGGACAGTTGTATTAAACCTTCTTTAGACAACTGAGTCTAAC
> T V L V G P T P V N I I G R N L L T Q I

8219 GTTGACTTTAAATTCCCCATTAGTCCATTGAAACTGTACCAGTAAATAAAGCCAG
CAACATGAAATTTAAAGGGGTAATCAGGATAACTTTGACATGGTTCATTTTAAATTTCGGTC

FIGURE 3 - CONTINUED

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>G C T L N F P I S P I E T V P V K L K P
 8279 GAATGGATGGCCCAAAGTTAAGCAATGGCCATTGACAGAGAGAAAATAAAGCATTAG
 CTTACCTACCGGGTTTTCATTCGTTACCGGTAACTGTCTTCTTTTATTTTCGTAATC
 >G M D G P K V K Q W P L T E E K I K A L
 8339 TAGAGATATGTACAGAAATGGAAAAGGAAGGAAAATTTCAAAAATTTGGGCCCTGAAAATC
 ATCTCTATACATGTCTTTACCTTTTCCCTTCCCTTTTAAAGTTTAAACCCGGACTTTTAG
 >V E I C T E M E K E G K I S K I G P E N
 8399 CATACAATACTCCAGTATTGTCTATAAGAAAAGACAGTACTAAATGGAGAAAACCTAG
 GTATGTTATGAGGTCATAAACGATATTCTTTTCTGTCTCATGATTTACCTCTTTTGATC
 >P Y N T P V F A I K K K D S T K W R K L
 8459 TAGATTTACAGAGAACTTAATAAAGAACTCAAGACTTCTGGGAAGTTCAGTTAGGAATAC
 ATCTAAAGTCTCTTGAATTATTTTCTTGAGTTCTGAAGACCCCTTCAAGTCAATCCTTATG
 >V D F R E L N K R T Q D F W E V Q L G I
 8519 CACACCCCGCAGGGTTAAAAAGAAAATCAGTAAACAGTATTGGATGTGGGTGATGCAT
 GTGTGGGGCGTCCCAATTTTCTTTTCTTTTAGTCATTGTCTATAACCTACACCCACTACGTA
 >P H P A G L K K K K S V T V L D V G D A

FIGURE 3 - CONTINUED

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8579 ACTTTTCAGTTCCTTAGATAAAGACTTTTAGAAAGTATACTGCATTTACCATACCTAGTA
TGAAAAGTCAAGGAATCTATTTCTGAAATCTTTTCATATGACGTAAATGGTATGGATCAT
>Y F S V P L D K D F R K Y T A F T I P S

8639 TAAACAATGAGACACCAAGGATTAGATATCAGTACAATGTGCTGCCACAGGGATGGAAG
ATTTGTTACTCTGTGGTCCCTAATCTATAGTCATGTACACGACGGTGTCCCTACCTTTC
>I N N E T P G I R Y Q Y N V L P Q G W K

8699 GATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAAACAGA
CTAGTGTGTCGTTATAAGGTTTCATCTGACTGTGTTTGTAGAACTCTCGGAAAAATCTTTGTCT
>G S P A I F Q S S M T K I L E P F R K Q

8759 ATCCAGACATAGTTATCTATCAATACATGGATGATTGTGTATGTAGGATCTGACTTAGAAA
TAGGTCTGTATCAATAGATAGTATTGTACCTACTAAACATACATCCTAGACTGAATCTTT
>N P D I V I Y Q Y M D D L Y V G S D L E

8819 TAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAGCATCTGTTGAGGTGGGATTTA
ATCCCGTCGTATCTTGTGTTTATCTCCTTGACTCTGTCGTAGACAACTCCACCCCTAAAT
>I G Q H R T K I E E L R Q H L L R W G F

FIGURE 3 - CONTINUED

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8879 CCACACCAGACAAAACATCAGAAAGAACCTCCATTCTTTGGATGGTTATGAACCTCC
GGTGTGGTCTGTTTTTTTGTAGTCTTTCTTGGAGGTAAGGAAACCTACCCAAATACCTTGAGG
>T T P D K K H Q K E P P F L W M G Y E L

8939 ATCCTGATAAATGGACAGTACAGCCTATAATGCTGCCAGAAAAGACAGCTGGACTGTCA
TAGGACTATTTACCTGTGTCATGTCGGATATTACGACGGTCTTTTCTGTGACCTGACAGT
>H P D K W T V Q P I M L P E K D S W T V

8999 ATGACATACAGAAGTTAGTGGGAAATTTGAATTGGGCAAGTCAGATTTATGCAGGGATTAA
TACTGTATGTCTTCAATCACCCCTTTTAACTTAACCCGTTTCAGTCTAAATACGTCCCTAAT
>N D I Q K L V G K L N W A S Q I Y A G I

9059 AAGTAAAGCAGTTATGTAACTCCTTAGAGGAACCAAGCACTAACAGAAATAATACCAC
TTTCATTTTCGTCAATACATTTGAGGAATCTCCTTGGTTTCGTGATTGTCTTCATTATGGTG
>K V K Q L C K L L R G T K A L T E V I P

9119 TAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAGAACAGTAC
ATTGTCTTCTTCGTCTCGATCTTGACCGTCTTTTGTCCCTCTAAGATTTCCTTGGTCAATG
>L T E E A E L E L A E N R E I L K E P V

9179 ATGAAGTATATTATGACCCATCAAAAGACTTAGTAGCAGAAAATACAGAAGCAGGGGCAAG

FIGURE 3 - CONTINUED

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TACTTCATAATACTGGGTAGTTTCTGAATCATCGTCTTTATGTCTTCGTCCTCCCGTTTC
>H E V Y Y D P S K D L V A E I Q K Q G Q

9239 GCCAATGGACATATCAAATTTATCAAGAGCCATTAAATACTGAATAACAGGAAAGTATG
CGGTTACCTGTATAGTTTAAATAGTTCTCGGTAAATTTTAGACTTTTGTCTTTCATAC
>G Q W T Y Q I Y Q E P F K N L K T G K Y

9299 CAAGGATAGGGTGCCACACTAATGATGTAAACAGTTAACAGAGGCAGTGCAAAAAG
GTTCCCTACTCCCCACGGGTGTGATTACTACATTTTGTCAATTGTCTCCGTCACGTTTTC
>A R M R G A H T N D V K Q L T E A V Q K

9359 TATCCACAGAAAGCATAGTAATATGGGAAAGATTCTCTAAATTTAAACTACCCATACAAA
ATAGGTGTCTTTCGTATCATTTATACCCCTTCTTAAGGATTTAAATTTGATGGGTATGTTT
>V S T E S I V I W G K I P K F K L P I Q

9419 AGGAAACATGGGAAGCATGGTGGATGGAGTATTGGCAAGCTACCTGGATTCTGAGTGGG
TCCTTTGTACCCCTTCGTACCACCTACCTCATAAACCGTTTCGATGGACCTAAGGACTCACCC
>K E T W E A W W M E Y W Q A T W I P E W

9479 AGTTTGTCAATACCCCTCCCTTAGTGAAATATGGTACCAGTTAGAGAAAGAACCCATAG
TCAAACAGTTATGGGAGGGAATCACTTTAATACCATGGTCAATCTCTTCTTGGGTATC

FIGURE 3 - CONTINUED

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>E F V N T P P L V K L W Y Q L E K E P I

9539 TAGGAGCAGAAACTTTCTATGTAGATGGGCAGCTAATAGGAGACTAAATTAGGAAAAG
ATCCTCGTCTTTGAAAGATACATCTACCCCGTCGATATCCCTCTGATTTAATCCTTTTC
>V G A E T F Y V D G A A N R E T K L G K

9599 CAGGATATGTTACTGACAGAGGAAGACAAAAGTTGTCTCCATAGCTGACACAACAATC
GTCCATATACAATGACTGTCTCCTTCTGTGTTTTCAACAGAGGTATCGACTGTGTGTTTAG
>A G Y V T D R G R Q K V V S I A D T T N

9659 AGAAGACTGAATTACAAGCAATTTCATCTAGCTTTGCAGGATTTCGGGATTAGAAGTAAACA
TCTTCTGACTTAATGTTTCGTTAAGTAGATCGAAACGTCTAAGCCCTAATCTTCATTTGT
>Q K T E L Q A I H L A L Q D S G L E V N

9719 TAGTAAACAGACTCACAATAATGCAATTAGGAATCATTCAGCACAAACCAGATAAGAGTGAAT
ATCATTTGTCGAGTGTATACGTAATCCTTAGTAAGTTTCGTTGGTCTATTTCTCACTTA
>I V T D S Q Y A L G I I Q A Q P D K S E

9779 CAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAGGAAAGGTCTACCTGGCAT
GTCTCAATCAGTCAGTTTATATCTCGTCAATTATTTTTCCTTTTCCAGATGGACCGTA
>S E L V S Q I I E Q L I K K E K V Y L A

FIGURE 3 - CONTINUED

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9839 GGGTACCAGCACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTAGTCAGTGCCTG
CCCATGGTCGTGTTTCTTAAACCTCTTACTTGTTCATCTATTAAATCAGTCACCGAC
>W V P A H K G I G G N E Q V D K L V S A

9899 GAATCAGGAAAGTACTATTTTTGAATGGAATAGATAAGGCCCAAGAACATGAGAAAT
CTTAGTCCTTTTCATGATAAATAAATTACCTTATCTATTCCGGTCTTCTTGTACTCTTTA
>G I R K V L F L N G I D K A Q E E H E K

9959 ATCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACTGCCACCTGTAGTACAAAG
TAGTGTCAATTAACTCTCGTTACCGATCACTAAATTTGGACGGTGGACATCATCGTTTTC
>Y H S N W R A M A S D F N L P P V A K

10019 AAATAGTAGCCAGCTGTGATAAATGTCAAGCTAAAGGAGAGCCATGCATGGACAAGTAG
TTTATCATCGGTCGACACTATTTTACAGTCGATTTTCTCTTCGGTACGTACCTGTTCATC
>E I V A S C D K C Q L K G E A M H G Q V

10079 ACTGTAGTCCAGGAATATGGCAACTAGATTGTACACATCTAGAGGAAAATTATCCTGG
TGACATCAGGTCCTTATACCGTTGATCTAACATGTGTAGATCTTCTCTTTTAAATAGGACC
>D C S P G I W Q L D C T H L E G K I I L

FIGURE 3 - CONTINUED

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10139 TAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAAGTTATTCAGCAGAGACAGGGC
ATCGTCAAGTACATCGGTACCTATATATCTTCGTCTTCAATAAGGTCGTCTGTCTCCCG
>V A V H V A S G Y I E A E V I P A E T G

10199 AGGAAACAGCATAATTTCTCTTAAATTAAGCAGGAAGATGGCCAGTAAACAAATACATA
TCCTTTGTCTGTATAAAGAGAAATTTTAATCGTCCTTCTACCGGTCAATTTTGTATGTAT
>Q E T A Y F L L K L A G R W P V K T I H

10259 CAGACAAATGGCAGCAAATTTCCACCAGTACTACGGTTAAGGCCGCCCTGTTGGTGCGCAGGGA
GTCTGTACCGTCGTTAAAGTGGTCAATGATGCCAATTCGGCGGACAAACCCGTCCTT
>T D N G S N F T S T V K A A C W A G

10319 TCAAGCAGGAATTTGGCATTTCCCTACAAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGA
AGTTCTGTCCTTAAACCGTAAGGATGTTAGGGGTTTCAGTTCTCTCATCATCTTAGATACT
>I K Q E F G I P Y N P Q S Q G V V E S M

10379 ATAAATGAATTAAAGAAATTAAGACAGGTAAGAGATCAGGCTGAACACCTTAAGACAG
TATTACTTAATTTCTTTTAATATCCTGTCCATTCTCTAGTCCGACTTGTGGAATTTCTGTC
>N N E L K K I I G Q V R D Q A E H L K T

10439 CAGTACAAATGGCAGTATTCATCCACAAATTTTAAAGAAAGGGGGATTTGGGGGATACA

FIGURE 3 - CONTINUED

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GTCAATGTTTACCGTCAATAAGTAGGTGTTAAAAATTTTCTTTTCCCCCTAACCCCTATGT
>A V Q M A V F I H N F K R K G G I G G Y

10499 GTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAACTAAAGAACTACAAA
CACGTCCCTTTTCTTATCATCTGTATTATCGTTGCTGTATGTTTGAATTTCTTGATGTTT
>S A G E R I V D I I A T D I Q T K E L Q

10559 AGCAAAATTACAAAAATTTTCGGGTTTATTACAGGACAACAAAGATCCCCTTT
TCGTTTAATGTTTAAAGTTTAAAGCCCAATAATGTCCCTGTTGTTTCTAGGGGAAA
>K Q I T K I Q N F R V Y Y R D N K D P L

10619 GGAAGGACCAGCAAGCTTCTCTGAAAGGTGAAGGGCAGTAGTAATACAAGATAATA
CCTTTCTGTCGTTTCGAAGAGACCTTTCCACTTCCCGTCATCATTAATGTTCTATTAT
>W K G P A K L L W K G E G A V V I Q D N

10679 GTGACATAAAAGTAGTCCAAAGAAGAAAGCAAAATCATTAGGGAATTATGGAAAACAGA
CACTGTATTTTTCATCACGGTTCTTCTTTTCGTTTTTTAGTAATCCCTAATACCTTTTGTCT
>S D I K V V P R R K A K I I R D Y G K Q

10739 TGGCAGGTGATGTTGTGGCAAGTAGACAGGATGAGGATTAGaaacatggaaaagtta
ACCGTCCACTACTAAACACACCGTTTCATCTGTCTCTACTCTCTAATCTgtaccttttcaaat

FIGURE 3 - CONTINUED

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>M A G D D C V A S R Q D E D •

10799 gtaaacaccatagggtcgactgcagaagctccatggggagctctttagtgtaataaat
catttgtggtatccagctgacgtcttcgaagggtaccctcgagaaatcacattattta

10859 ttaataaaatattgacaaaaatagttaaatgaatatatgaaagtacattatcacacggaATG
aattatttataaactgttttatcaatttacttatatactttcatgtaatatgtgcctTAC

10919 GAGTTCGATATTAGTTCTTGCAGAAATGATATATTCTGTCTCGAACAAATATCACTTTGTT
CTCAAGCTATAATCAAGAACGTCCTTACTATATAAGACAAGAGCTTGTATTATAGTGAAACAA

10979 ACTGATAATCGTTATAACAACCATATCAAAAATTAGAAATTATATTACTGTTTAAAA
TGACTATTAGCAATATTGTTGGTATTAGTTTTTAAATCTTAATAATAATGACAAATTTT

Fowlpox virus 3' flanking region of insertion site (in upper case)
→

11039 GATTCTACGATAAGAAATATCCGTACAGGTTTGTCTGAAATTCACCTTGTAGATAC
CTAAGATGCTATTCTTTATAGGCATGTCCAAACAAAGACTTTAAGTGAACATTCCTATG

11099 ATAATTAACAAATTCAGGGGAAAATCTTTACAAAATTAGTATAGAAGCTATAGATATA
TATTAATTGTTAAGTCCCCCTTTTAGAAAATGTTTAAATCATATCTTCGATATCTATAT

FIGURE 3 - CONTINUED

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11159 TCAAAAGGTAGACAACAATAATCAGAACCTAATTTTATTATCAAAAAATTAAAAATATAA
AGTTTCCCATCTGTTGTTTATTAGTCTTGGATTAAAAAATAAGTTTTTTAAATTTATATAT
11219 ATAAAAATGAAAAATAACTTGTATGAAGAAAAAATGAACATGAGTAAGAAACAAGTAAAAA
TATTTTACTTTTTTATTGAACATACTTCTTTTATTACTTGTACTCAATCTTTGTTTCATTTTT
11279 CTCAAAGTAAATGTAATAATAACGCATCTAGATTTACATGCCCTGGATCGCGTGCA
GAGTTTCATTTACATTATTATTGCGTAGATCTAAATGTACGGACCTACGCCACGT

FIGURE 3 - CONTINUED

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